Remarks:

In the Office Action dated August 5, 2004, claims 49-66, in the above-identified U.S. patent application were rejected. Reconsideration of the rejections is respectfully requested in view of the above amendments and the following remarks. Claims 49-66 remain in this application, claims 1-48 have been canceled.

Claim 49 was rejected under 35 USC §103(a) as unpatentable over Celeste, Ben-Bassat, Hirel and Georgiou. Applicants respectfully point out that Celeste was not able to prove bone/cartilage-inducing activity even for mature MP52. There is even less indication for short fragments (from which a reduced interaction with ECM is to be expected as discussed in applicant's prior response). Page 5 of the Office Action infers from the disclosure of Georgiou that it is particularly desirable to obtain a "natural product" since differences in a single amino acid could be detrimental to a patient. At the top of page 1240 Georgiou states: "The production of proteins that are exactly identical to the authentic product is particularly desirable in the pharmaceutical industry.". This suggests that Met at the N-terminus must be removed before Ala, but advises against removing Ala also. In contrast to the statements in the office action that Ala-Pro-MP52 and Pro-MP52 are natural products, applicants point out that only Ala-Pro-MP52 is a naturally occurring protein and thus is the only one which is the same as the "authentic protein" as meant by Georgiou. The formation of Pro-MP52 only takes place with the artificial expression of the mature MP52 in E.coli. MP52 does not normally occur in E.coli and thus cannot be expressed in this form in nature. In

nature, MP52 is only expressed in eukaryotes and in this case always as a precursor protein, from which the mature part is cleaved first. The cleavage of the mature part does not take place via methionyl aminopeptidase, the enzyme which cleaves methionine at the N-terminus, but via another enzyme which specifically recognizes the cleavage site RXXR typical for the TGF-β superfamily. The cleavage site of MP52 is the sequence RRKRRAP-MP52, from which mature MP52 with Ala-Pro-MP52 and because of the double occurrence of the motif RXXR in that sequence, also some (to a lesser extent) of Arg-Ala-Pro-MP52, is obtained in eukaryotes. The cleavage of the RXXR sequence, however, clearly cannot lead to Pro-MP52 since the cleavage is effected after an Arg (R) and not an Ala (A). Thus, it is clear that Pro-MP52 is not a natural product. This product, which is not natural, would be inappropriate for the pharmaceutical industry according to Georgiou.

The office action also states that the references do not describe the same methods but when using a "product-by-process" claim format, new or unexpected properties must be present in the product. Applicants point out that Hirel and Ben-Bassat indicate that a proline at the third position can disturb the efficiency of the methionine aminopeptidase in E.coli and that hybrid forms including a missing Ala can occur in a protein starting with Met-Ala-Pro. As a proposed solution, Ben-Bassat only indicates that the methionine aminopeptidase must be overexpressed (in vivo) in E.coli at the same time or used in vitro on the expressed purified protein in order to cleave the remaining Met at the N-terminus. Such a method has disadvantages such as additional contamination of the product by an enzyme which has to be removed by a further

purification step. Furthermore, it cannot be assumed that 100% cleavage of methionine will be obtained simply by increasing the amount of enzymes. Ben-Bassat indicates that by using the enzyme, a considerable improvement can be achieved, however, in a significant proportion, methionine still remains at the N-terminus (see page 755 above the discussion: "*It is interesting to note that a small fraction of the recombinant proteins, after being exposed to MAP either in vitro or in vivo, still retained their terminal methionines (Table 4))*". Table 4 shows that 6% in vitro and 5% in vivo of Met-Ala-Pro-Ile-2 possess an N-terminal methionine **after** treatment with MAP. Thus, some methionine remains despite the disadvantageous use of increased amounts of enzyme. Even when using additional MAP the problem remains that both Ala-Pro-MP52 and Pro-MP52 are present. Thus, a further reference, Tonouchi, must be taken into consideration, wherein it is described that Ala can be removed by the aminopeptidase P. This, however, means an additional treatment step which further deviates from the solution of the present invention. In addition, contamination can be expected to remain in the protein despite the aminopeptidase P. Furthermore, aminopeptidase P specifically cleaves the N-terminal amino acid only if Pro is present at the second position. In the case of Met-Ala-Pro-MP52 no cleavage of Met-Ala would be effected. The contamination with Met-Ala-Pro-MP52, which is to be expected even after MAP-treatment according to Ben-Bassat will remain despite the application of the aminopeptidase P of Tonouchi. Such contamination is avoided by the present invention and thus applicants contend that the presently claimed product is

different from the prior art product.

The process of the present application unexpectedly results in the expression of Pro-MP52 alone, i.e. completely without Met-Ala-Pro-MP52 or Ala-Pro-MP52 in a pharmaceutically usable pure product and additionally in an amount sufficient for industrial scale. However, Devlin suggests potential difficulties. G-CSF is expressed in E.coli therein as Met-Thr-Pro-GCSF with an inefficient removal of the methionine at the N-terminus. After removal of Thr, Met-Pro-GCSF is expressed wherein Met is efficiently removed and Pro-GCSF arises. Applicants point out page 20, column 2 where it is mentioned that the N-terminal Met is cleaved most effectively if a *"gyration of 1.22A or smaller (e.g., Gly or Ala)"* is present and on the other side, that the cleavage is inefficient at 1.43A and bigger. The cleavage is inconsistent with amino acids with intermediate *"radii of gyration (e.g., Thr,* **Pro,** or *Val)".* The inconsistency is ascribed to the further amino acids nearby the N-terminus and their tertiary structure. In view of this, one skilled in the art would assume in the case of MP52 that it is of questionable use if Pro is in the second position after Met. A certain probability exists that the cleavage would not be effective for obtaining a pure pharmaceutical product. A contaminated product would be detrimental for patients due to the additional Met. Similar conclusions can be gathered from Hirel (1989), Table 1 for the expression in E.coli with Pro at the second position where only an efficiency of methionine cleavage of 88.2% is obtained. Since MP52, like other BMPs, is used in relatively high dosages (approx. 1 mg), 12% of the remaining methionine at the N-terminus would not be acceptable as concluded by Georgiou. Thus, only by means of the present invention can an

efficient cleavage of methionine before proline occur in the case of Pro-MP52.

In addition, applicants point out that it would not have been obvious to express MP52 starting with Pro after Met in E.coli on an industrial scale. It was known from Looman (1987) that the translation initiation rate was strongly dependent on the second codon (see Hirel, page 8249, column 1 which states that "*In contrast, the production of the various hybrid proteins appeared highly sensitive to the second codon substitution. According to the second codon efficiency rule of Looman at al. (23) established with the lac Z gene, the translation initiation rate of a gene strongly depends on the nature of its second codon*"). It can be gathered from Figure 3 that proline has a relative efficiency near 0 according to Looman, whereas Ala is above 2.

For the industrial production of a pharmaceutical product, one skilled in the art tries to choose conditions which allow a high expression. According to Looman, a high translation efficiency is to be expected only with Ala at the second position and not with proline at the second position. The expectation of a bad translation efficiency for Met-Pro-MP52 (from Hirel/Looman), linked with the known inconsistency when methionine is cleaved with the subsequent Pro, i.e. an expected contamination of Pro-MP52 with Met-Pro-MP52 (cf. Hirel/Devlin), and in view of Georgiou's conclusions regarding an "authentic protein", one skilled in the art would not be motivated to express Pro-MP52 in E.coli for pharmaceutical purposes on an industrial scale.

The unobviousness of the process of the present invention is on the one hand the complete avoidance of the hybrid form for MP52 and on the other hand the

unexpectedly high expression rate of Pro-MP52, which is necessary for industrial production, combined with an efficient cleavage of methionine which was unexpected. The aim of the present invention is the production of the protein in large-scale or industrial scale, which was surprisingly achieved with the expression of Pro-MP52. In view of the above discussion and amendments, applicants request that this rejection be withdrawn.

Claim 49 was rejected under 35 USC §103(a) as obvious over Celeste, Ben-Bassat, Hirel, Georgiou, Thompson and Tonouchi. Claims 49 and 50 were rejected under 35 USC §103(a) as obvious over Celeste, Ben-Bassat, Hirel, Georgiou, Thompsen and Tonouchi further in view of Hotten and Cerletti. As discussed above, Met-Ala-Pro-MP52 and Ala-Pro-MP52 are not expressed in the present invention. Therefore, it is not necessary to use aminopeptidase P for the removal of Ala when using the present invention. Claim 49 has been amended to clarify that proteins according to SEQ ID NO: 1 with either a) an Ala, or b) a Met-Ala at the N-terminus are not expressed and are not present in the isolated protein. In view of the above discussion and amendments, applicants request that this rejection be withdrawn.

Claims 49-60, 63 and 66 were rejected under 35 USC §103(a) as obvious over Celeste, Ben-Bassat, Hirel, Georgiou, Thompson and Tonouchi further in view of Hotten, Cerletti and Neidhardt. Neidhardt was cited for the disclosure of a pharmaceutical composition comprising MP52, but does not cure the deficiencies in the other references regarding the expression of a protein according to SEQ ID NO:1 without the expression of proteins according to SEQ

ID NO:1 with an Ala, or Met and Ala at the N-terminus. As discussed above, claim 49 has been amended to clarify that proteins according to SEQ ID NO: 1 with either a) an Ala, or b) a Met-Ala at the N-terminus are not expressed and are not present in the isolated protein. In view of these amendments and the above discussion, applicants request that this rejection be withdrawn.

Claims 49-60 and 63-66 were rejected under 35 USC §103(a) as obvious over Celeste, Ben-Bassat, Hirel, Georgiou, Thompson and Tonouchi further in view of Hotten 2, Cerletti, Neidhardt, Hotten A and Chen. Chen describes different matrices. Chen does not cure the deficiencies in the other references regarding the expression of a protein according to SEQ ID NO:1 without the expression of proteins according to SEQ ID NO:1 with an Ala, or Met and Ala at the N-terminus. Though the combination of prior art references are argued to suggest removal of proteins according to SEQ ID NO:1 with an Ala, or Met and Ala at the N-terminus, none of these references suggests that it is possible to express proteins according to SEQ ID NO:1 without expressing proteins with an Ala, or Met and Ala at the N-terminus. As discussed above additional steps are required when using the prior art processes to remove the unwanted proteins. In view of this, applicants contend that the combination of cited references does not render the presently claimed invention obvious and request that this rejection be withdrawn.

Claims 49-51 and 61-62 were rejected under 35 USC §103(a) as obvious over Celeste, Ben-Bassat, Hirel, Georgiou, Thompson and Tonouchi further in view of Hotten 2, Cerletti, Neidhardt, Ron and Avis. Applicant's respectfully point out that

Ron's invention consists of a mixture of "osteogenic proteins, a porous particulate polymer matrix and an osteogenic protein-sequestering amount of blood" (column 1 of the Summary). These three components together form a deformable implant. The pharmaceutical composition in Ron is implanted and not injected. Even if single components like the osteogenic factors can be stored in a more soluble lyophilized form, Ron's actual pharmaceutical composition for application in a patient is not injected. Clotted blood is not injectable, however, Ron added blood in such a way that there would be sufficient time for clotting before application in a patient (column 4, bottom: "*The blood to be used in the formulation is admixed at a time prior to use sufficient to allow clotting*"). Thus, Ron does not suggest a product suitable for injection in a patient. Avis generally discusses the advantages of lyophilization but does not indicate that the presently claimed protein can be prepared and lyophilized. In addition, neither Ron nor Avis cure the deficiencies in the other references regarding the expression of a protein according to SEQ ID NO:1 without the expression of proteins according to SEQ ID NO:1 with an Ala, or Met and Ala at the N-terminus. In view of this, applicants contend that the combination of cited references does not render the presently claimed invention obvious and request that this rejection be withdrawn.

Claims 49-51 and 60 were rejected under 35 USC §103(a) as obvious over Celeste, Ben-Bassat, Hirel, Georgiou, Thompson and Tonouchi further in view of Hotten 2, Cerletti, Neidhardt, and Oppermann. Oppermann mixes osteogenic proteins in saline by means of a vortex together with a matrix in order to obtain an osteogenic material. Osteogenic proteins in saline are additionally mixed with a matrix

before application. The matrices consist of porous particles of different materials as mentioned at the bottom of column 11. These materials are not injected into the bloodstream for systemic use since such use would result in an occlusion of the veins. The complete Oppermann document aims at producing an **implant** made of osteogenic protein and a matrix (cf. e.g. column 5, Summary of the Invention: *"This invention provides osteogenic proteins and devices which, when implanted in a mammalian body, can induce at the locus of the* implant the *full development cascade of endochondral bone* formation...". Oppermann does not suggest that osteogenic proteins are active in saline alone, i.e. without a matrix, nor does he suggest that osteogenic proteins can be used systemically. In addition, Oppermann does not cure the deficiencies in the other references regarding the expression of a protein according to SEQ ID NO:1 without the expression of proteins according to SEQ ID NO:1 with an Ala, or Met and Ala at the N-terminus. In view of this, applicants contend that the combination of cited references does not render the presently claimed invention obvious and request that this rejection be withdrawn.

Claims 49-66 were rejected under 35 USC §112, second paragraph, as indefinite. Claim 49 has been amended to clarify that proteins according to SEQ ID NO:1 with either a) an ala or b) a Met-Ala at the N-terminus are not expressed and are not present in the isolated protein. In view of these amendments, applicants request that this rejection be withdrawn.

Claim 49 was provisionally rejected under the judicially created doctrine of obviousness type double patenting as unpatentable over claim 14 of co-pending

application serial no. 09/701,121. Co-pending application serial no. 09/701,121 has been abandoned for failure to pay the issue fee. In view of this abandonment, this rejection is moot.

Claim 49 was provisionally rejected under the judicially created doctrine of obviousness type double patenting as unpatentable over claim 4 of co-pending application serial no. 10/751,451. Applicants respectfully point out that claim 4 of co-pending application no. 10/751451 requires a protein with an amino acid sequence according to SEQ ID NO:2. SEQ ID NO. 2 in 10/751,451 is different from SEQ ID NO:1 in the present application in that the Cys residue at position 83 has been replaced with an Ala residue. Thus, claim 4 in co-pending application no. 10/751451 does not encompass claim 49 in the present application. In view of this, applicants request that this rejection be withdrawn.

Claims 49-66 were provisionally rejected under the judicially created doctrine of obviousness type double patenting as unpatentable over claims 1-7 of co-pending application serial no. 10/365,231. Co-pending application serial no. 10/365,231 is a divisional application of the present application. There has not yet been an examination on the merits or a restriction requirement. Claims which are different from the claims in the present application will be pursued in the divisional application. In view of this, applicants request that this provisional rejection be held in abeyance until an examination on the merits or a restriction requirement has been made in co-pending application serial no. 10/365,231.

Applicants respectfully submit that all of claims 49-66 are now in condition for allowance. If it is believed that the application is not in condition for

allowance, it is respectfully requested that the undersigned attorney be contacted at the telephone number below.

In the event this paper is not considered to be timely filed, the Applicant respectfully petitions for an appropriate extension of time. Any fee for such an extension together with any additional fees that may be due with respect to this paper, may be charged to Counsel's Deposit Account No. 02-2135.

Respectfully submitted,

By _____

Monica Chin Kitts/Robert B. Murray, 22,980
Attorney for Applicants
Registration No. 36,105
ROTHWELL, FIGG, ERNST & MANBECK, p.c.
Suite 800, 1425 K Street, N.W.
Washington, D.C. 20005
Telephone: (202)783-6040